A PRELIMINARY NOTE ON TETRAMITUS, A STAGE IN THE LIFE CYCLE OF A COPROZOIC AMOEBA

BY MARTHA BUNTING

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF PENNSYLVANIA

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In 1920 a study of the Entamoeba of the rat was begun and in a culture on artificial medium a number of Coprozoic amoeba appeared, one of which exhibited a flagellate phase which seems to be identical with Tetramitus rostratus Perty.[1]

The appearance of flagellate phases in cultures of Coprozoic amoeba (Whitmore,[2] etc.) as well as of soil amoeba (Kofoid,[3] Wilson,[4] etc.) has been recorded a number of times, but the flagellates which appeared were relatively simple in organization and very transitory in occurrence. Furthermore, some of the simpler flagellates have been observed (Pascher[5]) to lose their flagella and become amoeboid. In the case here described, however, there is a transformation of a simple amoeba into a relatively complex flagellate which multiplies for several days then returns to the amoeboid condition and becomes encysted. Tetramitus rostratus has been studied by a number of investigators since its discovery in 1852, by Perty,[1] yet no one has given a full account of its life history. The lack of cysts in previous accounts, mentioned by Dobell and O'Connor,[6] is explained by the transformation into an amoeba before encystment. It is believed that this animal presents the extreme in transformations of this sort and serves to emphasize the close relationship between amoebae and flagellates, and the need for careful studies of life cycles, in pure cultures where possible, in investigations of coprozoic and other Protozoa. It is proposed to record at this time a brief account of the life history of this amoebo-flagellate reserving the more extended discussion for future publication.

The amoeba in question was first observed on November 5, 1920 in coecal material which had been taken from the rat and placed in sterile physiological salt solution on October 28, 1920. This is known as culture 10. After discovery of the amoeba, transplants were immediately made in various media. The amoebae were subcultured several times on solid media over a period of about two months, then two cultures were made in fluid media. In one the formula given by Sellards,[7] namely, 1.0 gr. Witte's peptone, 0.5 gr. lactose, 1000 cc. distilled water was used; in another, dextrose was substituted for lactose in Sellards' formula. The medium containing dextrose proved to be much more favorable for the amoeba under consideration. In these liquid media a flagellate appeared which is thought to be Tetramitus rostratus Perty.

In these cultures differences among the active amoebae as well as three types of cysts and the flagellate were noted, hence pure lines were started by the isolation method. Pure lines derived from culture 10 were designated G., I., J. and L.; of these G. and L. are of more special interest, the former being obtained from a single specimen of Tetramitus, the latter from a smooth, thin-walled cyst.

The cysts of cultures G. and L. revealed no microscopic differences either in the living or in the fixed and stained condition, and when transplants were made of the cysts in favorable media both active amoebae of limax type and Tetramitus developed. Pure lines started from an isolated amoeba, an isolated flagellate or an isolated cyst in liquid or semiliquid media at room temperature have always given the same results, i.e., smooth, thin-walled cysts, the active amoeba of limax type and Tetramitus rostratus.

Although the amoeba with the Tetramitus stage was obtained in cultures from the coecal contents of the rat, the presence of contractile vacuoles etc., indicated that this amoeba was not a parasite but belonged to the coprozoic protozoa as defined by Dobell and O'Connor.[6] Feeding experiments upon rats and mice were conducted to determine whether the amoeba and flagellate stages are present either in the small intestine or in the coecum of these animals. Prior to feeding these experimental animals their faeces was collected on filter paper and cultures made in liquid media; in a few cases the amoeba and tetramitus stage was already present. The nine rats and four mice used were killed and opened in one, two, three, and five days after feeding and microscopic examination made of the fresh contents of the coecum and small intestine. Neither active amoebae nor Tetramitus were found, but cysts, both degenerate and normal, were present in all the animals. Cultures were made of coecal contents in sterile liquid media which were studied the following day. The active amoeba of the type used in the feeding and Tetramitus were found in these cultures. The results of the feeding experiments indicate that the amoeba under cultivation belongs to the coprozoic Protozoa.

Much evidence that Tetramitus is a stage in the life history of this coprozoic amoeba was obtained from the study of the living animal and from fixed and stained cover glass preparations, yet to affirm this fact with certainty, many consecutive hours of many days have been spent in close observation. Much of this study has been conducted on hanging drops, made either from a single cyst isolated and placed in a drop of fresh liquid medium or a few cysts taken by a platinum loop from a culture containing only cysts and adding from two to three loops of fresh medium. All were placed on depression slides and ringed with hard vaseline. By this method the animals have been kept under observation during the entire

life cycle, a period of about four days. A brief description of the living animal during the stages of its life cycle will now be given.

1. *The Cyst:*—(fig. 1, A) *a*. Morphology. Commonly spherical, occasionally broadly oval, single, smooth, relatively thin wall; usually uninucleate; rarely binucleate; size ranging from 6μ to 18μ.

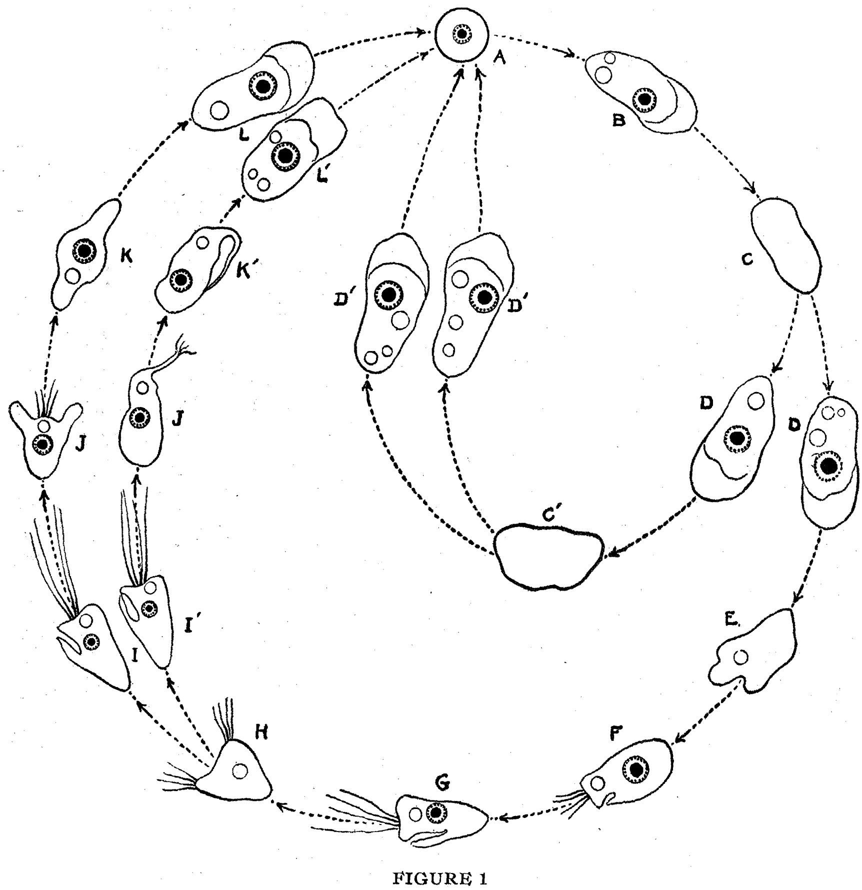

FIGURE 1

Diagram of life-cycle of coprozoic amoeba with Tetramitus as its flagellate phase. A—the cyst; B—vegetative amoeba; C, C′—division ("gel" state); D, D′—amoebae after division; E, F—phases in transformation of Tetramitus; G—fully formed Tetramitus; H—Tetramitus previous to division ("gel" state); I, I′—Tetramitus after division; J, K, L and J′, K′, L′—series of transformation stages, Tetramitus to amoeba.

b. Excystation. No empty cyst wall remains after the amoeba has emerged from the cyst, the wall apparently undergoing solution during the process of excystation.

2. *Amoeba:*—(fig. 1, B) *a.* Vegetative stage. Up to the present date the animal has always been observed to leave the cyst stage as an amoeba, never as a Tetramitus. The amoeba varies from 14μ to 48μ in length.

The rounded vesicular nucleus is usually 5μ to 6μ in diameter, and contains a caryosome 1μ to 2μ less in diameter; the pericaryosomal zone is usually clear but may contain a few granules. Peripheral chromatin on the nuclear wall in the form of granules can sometimes be observed in the living condition and generally in the fixed and stained specimens. The cytoplasm becomes sharply defined into a non-granular ectoplasm and granular endoplasm during the protrusion of pseudopodia. The amoeba generally moves by means of one broadly lobose pseudopodium, which however, may not be withdrawn before additional ones are formed. There is always one large contractile vacuole, augmented frequently by a few smaller ones.

b. Division. Gradually the rapid movement of the granules and of the nucleus through the endoplasm becomes slower, the pseudopodium is retracted, the granules clump together, then lose their identity so that the entire protoplasmic body becomes homogeneously refractive, and thus a "gel" state is formed during which the nucleus is obscured (fig. 1, C). The animal may remain quiescent in this state for from thirty seconds to several minutes, after which it suddenly elongates, constricts in the middle and divides into two daughter cells, meanwhile passing from the "gel" to the "sol" state (fig. 1, D, D′). The formation of a "gel" state during the process of division in higher animals has been noted in many papers by a number of observers, among whom are Chambers[8] and Heilbrunn.[9] Multiplication may continue for a number of generations without flagellation, or the amoeba may pass through a flagellate phase. Finally in each case the amoeba becomes encysted.

3. *Flagellate Phase:*—(fig. 1, G). Tetramitus varies much in size and in form as has been noted by Perty,[1] Fresenius,[10] Dallinger and Drysdale,[11] Stein,[12] and Klebs,[13] but the typical form is that described by them as "conical" with the pointed end directed posteriorly. The contractile vacuole and cytostome are located at the flattened anterior end which bears four flagella. The nucleus is slightly posterior to the contractile vacuole, and is similar in organization to that of the amoeba just described except that it is smaller measuring 3μ to 5μ in diameter. The animal is usually 14μ to 18μ in length and 7μ to 10μ in greatest width.

4. *Transformation of an Amoeba to Tetramitus:*—After the amoeba has passed through one or more generations by division, some of them may transform into flagellates. While the morphological changes may vary during the process, the results of one observation will now be given in detail. An amoeba retracted its pseudopodium, but changes in outline

continued slowly; the cytoplasm became homogeneously refractive and the nucleus was obscured probably due to a "gel" state (fig. 1, E). The contractile vacuole continued to pulsate slowly; an involution of the lateral margin near this organelle was formed which terminated in the protoplasmic body as a cleft. The animal now assumed an elongated somewhat pyriform shape, the margin near the vacuole being flattened. From this area flagellar activity began, and thus it became oriented as the anterior end. The nucleus, nearer the posterior end of the animal became demarcated from the homogeneously refractive cytoplasm (fig. 1, F). The animal began to move rapidly, undergoing many contortions, during which the cytoplasm became resolved into refractive granules suspended in a clear substance, the nucleus moved towards the anterior end and the pellicle became more firm so that the flagellate shape was assumed (fig. 1, G). The flagella had reached the average length and the characteristic rotation around the longitudinal axis began as the animal swam swiftly about.

6. *Division of the Flagellate:*—In a short time after the above described change the animal suddenly became broadly triangular in form (fig. 1, H), flagellar activity being observed at each angle limiting the anterior end. The animal then passed into the "gel" state and this was followed by longitudinal division (fig. 1, I, I′). The entire transformation, followed by fission in the above case, was completed in one hour, but these processes are not always so rapid. The flagellate may continue to multiply for several hours or even days commonly dividing into two by longitudinal fission but occasionally into three or four equal or unequal offspring.

7. *Transformation of Flagellate into an Amoeba:*—Eventually the Tetramitus transforms into an amoeba unless death prevents. The morphological changes which form this sequence are not always the same, but one set of observations will be given in detail. A Tetramitus suddenly shortened and became broadly triangular, two short processes were protruded from the angles of the flattened anterior end (fig. 1, J). Soon the body became rounded, the processes longer (fig. 1, K), and the movements slower resulting in but slight changes of location. Meanwhile the protoplasm exhibited external form changes marked by contortions of the body. The successive changes were very rapid and suggested that an effort was being made by the animal to free itself of flagella. Finally it became quiescent a few moments and then changed into the form of the typical amoeba which progressed by a pseudopodium of lobose type instead of by flagella (fig. I, L). Another series of changes are indicated by fig. 1, J′, K′ and L′. The transformation process is sometimes prolonged through many hours. The amoebae thus derived may multiply by division but ultimately they become encysted completing the life cycle.

The time periods occupied by the different stages in the life cycle at room temperature, as a rule, have been the following:—The cyst lives in a proper medium indefinitely. When cysts are planted on fresh media, amoebae may emerge in two or three hours and remain active from three to four days, rarely longer, and then encyst. The Tetramitus may appear in ten to twelve hours after transplantation of the cysts, continue numerous for two days and gradually disappear the third day as these become transformed into amoebae or die.

Exceptions to the above have occurred in two cultures in which activity of Tetramitus lasted for about a month. The study of the living animals together with the cytological examination of fixed and stained specimens from each culture revealed an increase in the number of nuclei to two, three and four, also the presence of giants and a greater proportion of animals of irregular shape.

While great numbers of these flagellates have been under observation for more than a year, no stages in the life history have been observed other than those described above. Binary fission for Tetramitus has been noted by Perty,[1] Dallinger and Drysdale,[11] Stein,[12] Klebs,[13] and Alexeieff.[14] Although no sexual stages nor spore formation such as described by Dallinger and Drysdale have been observed in these studies, nevertheless these observers did see the flagellate become partly amoeboid and a longer observation should have revealed to them the complete change to an amoeba.

In a life cycle of this kind, in the absence of sexual phenomena it is uncertain whether the amoeboid or the flagellate phase should be considered as the adult or dominant one, especially since non-sexual reproduction is extensive in both phases. A thorough study of the life cycles of other "amoebae" and other "flagellates" might reveal further examples of transformations of the kind here described.

In closing I wish to express my great indebtedness to Doctor D. H. Wenrich who has been ever ready to aid this investigation by participation and by fruitful suggestions. Also for the continued interest and aid of Professor Clarence E. McClung who has helped to surmount the many difficulties encountered and who, with the Graduate School of the University of Pennsylvania, has provided the facilities of the Zoölogical Laboratory for this research.

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A FURTHER NOTE ON THE AGE INDEX OF A POPULATION[1]

BY RAYMOND PEARL AND T. J. LEBLANC

SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS

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In an earlier paper in these PROCEEDINGS, Pearl[2] proposed the function

$$\phi = S\left\{\frac{\Delta^2}{P}\right\}(M - M_p)$$

as a single numerical index of the age distribution of a population. In this expression S indicates summation, for all age groups given in the original statistics, of the expression in brackets; Δ is the deviation, for each age group, of the percentage of the actual population in each age group from the percentage of the same group in the standard population of Glovers[3] Life Table, denoted by P; M = mean age of living population; M_p = mean age of persons in a stationary population unaffected by migration.

In connection with certain problems now under investigation in this laboratory it becomes a matter of importance to know how sensitive this age index is to change in the size of the age groups of the original statistics. Since its original proposal the index has been used in many studies in this laboratory, and the larger experience has strengthened our confidence in its reliability as an index of significant variations in the age constitutions of populations. But it has always been used hitherto with at least 6 to 8 age groups covering the life span. Suppose the original statistics furnish only 3 age classes for the entire life span. Will this age index ϕ then give a reliable picture of the significant variations in age distribution, as we pass from city to city, or county to county?

To test this point, the obvious thing to do is to determine the correlation between the age indices for n communities, on the basis of say 3 divisions of the life span, with the age indices for the same communities on the basis of say 6 divisions of the life span. If the correlation is high it will mean